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Influence of UVB radiation on the lethal and sublethal toxicity of dispersed crude oil to planktonic copepod nauplii

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HIGHLIGHTS

- Dispersed crude oil and dispersant Corexit 9500 are highly toxic to copepod nauplii.
- UVB radiation substantially increases the toxicity of crude oil to copepod nauplii.
- Dispersed crude oil cause sublethal effects on copepod nauplii, such as reduced growth, development and swimming activity.

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ABSTRACT

Toxic effects of petroleum to marine zooplankton have been generally investigated using dissolved petroleum hydrocarbons and in the absence of sunlight. In this study, we determined the influence of natural ultraviolet B (UVB) radiation on the lethal and sublethal toxicity of dispersed crude oil to naupliar stages of the planktonic copepods *Acartia tonsa*, *Temora turbinata* and *Pseudodiaptomus pelagicus*. Low concentrations of dispersed crude oil (1 μL L^{-1}) caused a significant reduction in survival, growth and swimming activity of copepod nauplii after 48 h of exposure. UVB radiation increased toxicity of dispersed crude oil by 1.3–3.8 times, depending on the experiment and measured variables. Ingestion of crude oil droplets may increase photoenhanced toxicity of crude oil to copepod nauplii by enhancing photosensitization. Photoenhanced sublethal toxicity was significantly higher when *T. turbinata* nauplii were exposed to dispersant-treated oil than crude oil alone, suggesting that chemical dispersion of crude oil may promote photoenhanced toxicity to marine zooplankton. Our results demonstrate that acute exposure to concentrations of dispersed crude oil and dispersant (Corexit 9500) commonly found in the sea after oil spills are highly toxic to copepod nauplii and that natural levels of UVB radiation substantially increase the toxicity of crude oil to these planktonic organisms. Overall, this study emphasizes the importance of considering sunlight in petroleum toxicological studies and models to better estimate the impact of crude oil spills on marine zooplankton.

1. Introduction

After a marine oil spill, crude oil is affected by a variety of abiotic and biotic processes that determine the fate and impact of petroleum pollution in marine ecosystems (National Research Council, 2003). One of the major abiotic factors affecting spilled crude oil is sunlight, mainly ultraviolet (UV) radiation (Garrett et al., 1998; Lee, 2003; Guipeng et al., 2006; Fathalla, 2007). UV radiation not only promotes photochemical degradation of crude oil (e.g. photooxidation) (Garrett et al., 1998; Lee, 2003), but may also increase the toxicity of petroleum pollution to marine organisms (Boese et al., 1997; Pelletier et al., 1997; Barron et al., 2003; Duesterloh et al., 2003; Almeda et al., 2013a). Some crude oil compounds, such as alkenes and polycyclic aromatic hydrocarbons (PAHs), strongly absorb UV light, inducing photo-enhanced toxicity of crude oil (i.e.

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increased crude oil toxicity in the presence of light) (Landrum et al., 1987; Kagan et al., 1990; Arfsten et al., 1996; Nicodem et al., 1997). Photo-enhanced toxicity may be caused by (1) photosensitization, where bioaccumulated petroleum compounds act as photoreceptors and transfer light energy to other surrounding biomolecules generating reactive oxygen species that cause cell damage, and/or (2) photomodification, where petroleum hydrocarbons are chemically transformed into more toxic compounds (Landrum et al., 1987; Kagan et al., 1990; Arfsten et al., 1996; Nicodem et al., 1997). However, most studies that have investigated the toxicity of crude oil were performed under artificial, fluorescent light, disregarding the influence of UV radiation on the toxicity of crude oil to marine organisms (Arfsten et al., 1996).

Among marine organisms, copepods are the dominant component of mesozooplankton communities (Longhurst, 1985; Humes, 1994). Planktonic copepods play a key role in pelagic systems, mediating the transfer of matter from primary producers to higher trophic levels and contributing to marine biochemical cycles (Banse, 1995; Alcaraz et al., 2010). Postembryonic development of planktonic copepods generally includes 11 larval stages, six naupliar stages (designated as nauplius I to nauplius VI, NI to NVI) and five copepodid stages (namely copepodid I to copepodid V, CI to CV) (Ferrari and Dahms, 2007). Copepod nauplii are considered the most abundant forms of metazoans on the planet (Björnberg, 1986; Fryer, 1986) and the main prey for many fish larvae (Last, 1980) contributing to the recruitment of commercially important fish stocks (Castonguay et al., 2008). However, despite the importance of copepods in marine systems, copepod nauplii are frequently neglected from environmental research (Björnberg, 1986), especially among eco-toxicological studies. In fact, we know little about the lethal and sublethal effects of dispersed crude oil and photenhanced toxicity on planktonic copepod nauplii, which is essential for understanding the impact of oil spills on copepod population dynamics and marine planktonic food webs.

During a marine crude oil spill, zooplankton are exposed to crude oil droplets generated by natural mixing or/and application of chemical dispersants (Forrester, 1971; Caneveri, 1978; Lichtenthaler and Daling, 1985; Delvigne and Sweeney, 1988). Dispersed crude oil droplets (1–100 μm) are frequently within the zooplankton prey size spectra (Hansen et al., 1984) and may be ingested by copepods and other planktonic organisms (Conover, 1971; Mackie et al., 1978; Gyllenburg, 1981; Lee et al., 2012; Almeda et al., 2014a, b, c). A recent study found that both feeding-current and ambush feeder copepod nauplii ingested dispersed crude oil (Almeda et al., 2014a). However, most toxicological studies on zooplankton have been conducted using the crude oil water soluble fraction (WSF), or certain mixed or individual petroleum hydrocarbons (Corner et al., 1976; Harris et al., 1977; Berdugo et al., 1997; Bejarano et al., 2006; Jiang et al., 2010, 2012) overlooking the influence of ingestion of crude oil droplets on the toxicity of crude oil to planktonic copepods. Similarly, photoenhanced toxicity of petroleum to marine animals has been mainly investigated using dissolved petroleum hydrocarbons (Boese et al., 1997; Pelletier et al., 1997; Barron et al., 2003) whereas the information on phototoxicity of crude oil to zooplankton is limited. Also, chemical dispersants (e.g. Corexit 9500A) used to treat oil spills, which can be toxic to planktonic organisms, particularly to larval stages (George-Ares and Clark, 2000; Goodbody-Gringley et al., 2013; Almeda et al., 2014b, d), may influence the photo-ehanhced toxicity of crude oil to aquatic animals (Barron et al., 2003). As far as we know, combined effects of chemical dispersants and UV radiation on crude oil toxicity to planktonic copepod nauplii have not been previously investigated.

Photoenhanced toxicity studies with crude oil or PAHs have usually been conducted using UVA and UVB simultaneously and, therefore, the relative contribution of different UV regions of the light spectrum to photoenhanced toxicity of petroleum is not well known (Boese et al., 1997; Pelletier et al., 1997; Barron et al., 2003; Duesterloh et al., 2003). In a previous study (Almeda et al., 2013a), we estimated the influence of UV/sunlight exposure on the toxicity of dispersed crude oil to natural copepod assemblages using 3 different light regimes: 1) the ambient full solar radiation spectrum (PAR + UVA + UVB), 2) the ambient full spectrum without UVB (PAR + UVA) and 3) no light (i.e. complete darkness). We found that PAR + UVA radiation did not significantly enhance the toxicity of dispersed crude oil to mesozooplankton after 48 h of exposure compared to dark incubations and that photo-enhanced toxicity of dispersed oil was due mainly to UVB exposure (Almeda et al., 2013a). Thus, although UVA or other light spectrum regions can enhance toxicity of petroleum hydrocarbons (Diamond et al., 2000; Barron et al., 2003), our previous results and those of other studies (Huovinen et al., 2001; Barron et al., 2003) suggest that UVB plays the dominant role in determining the magnitude of photoenhanced toxicity of crude oil to zooplankton. Understanding the effect of UVB radiation on the phototoxicty of crude oil is of particular interest because levels of UVB radiation have increased at the Earth's surface as a result of the stratospheric ozone depletion during the last decades (Madronich et al., 1998; McKenzie et al., 2007).

In the present study, we aim to 1) determine the toxicity of mechanically and/or chemically dispersed crude oil to copepod nauplii and 2) measure the influence of natural UVB radiation levels on toxicity of dispersed crude oil to copepod nauplii. To that end, we estimated the effects of dispersed crude oil on survival, growth rates and swimming behavior of copepod naupllii after 48 of exposure with and without natural UVB radiation. We used naupliar stages of the calanoid planktonic copepods *Temora turbinata*, *Acartia tonsa*, and *Pseudodiaptomus pelagicus* (Fig. 1). These species belong to some of the most representative copepod genera in estuarine and coastal subtropical and temperate waters, including the Gulf of Mexico, where these species are among the most common mesozooplankton (Razouls, 2005–2013). This study is particularly valuable for increasing our understanding of the potential impact of oil spills on zooplankton communities in the Gulf of Mexico, a region with a high risk for oil spills due to the intense oil production and transportation.

2. Material and methods

2.1. Experimental organisms

Zooplankton samples were collected from the Aransas Ship Channel near the University of Texas Marine Science Institute or from a nearby channel in Corpus Christi Bay (Port Aransas, Texas) using a plankton net (150 μm mesh, 50 cm diameter). Plankton samples from the Corpus Christi Bay channel were collected by towing the plankton net through the surface water, whereas samples from the Aransas Ship Channel were collected from surface waters by tying a plankton net to the University of Texas Marine Science Institute pier and allowing it to stream with the tidal current for approximately 5–10 min. Specimens of *A. tonsa* and *P. pelagicus* were isolated from samples collected in the Corpus Christi Bay Channel in July 2013. *T. turbinata* were isolated from zooplankton samples taken in September/October 2014 from the Aransas Ship Channel during flood tides from the Gulf of Mexico. Contents of collection buckets (cod ends) were poured in a cooler containing *in situ* unfiltered seawater until returning to the laboratory, where samples were lightly aerated.

Once in the laboratory, aliquots of the zooplankton samples were examined under a dissecting microscope and copepod adult stages of *T. turbinata*, *A. tonsa*, and *P. pelagicus* were identified and

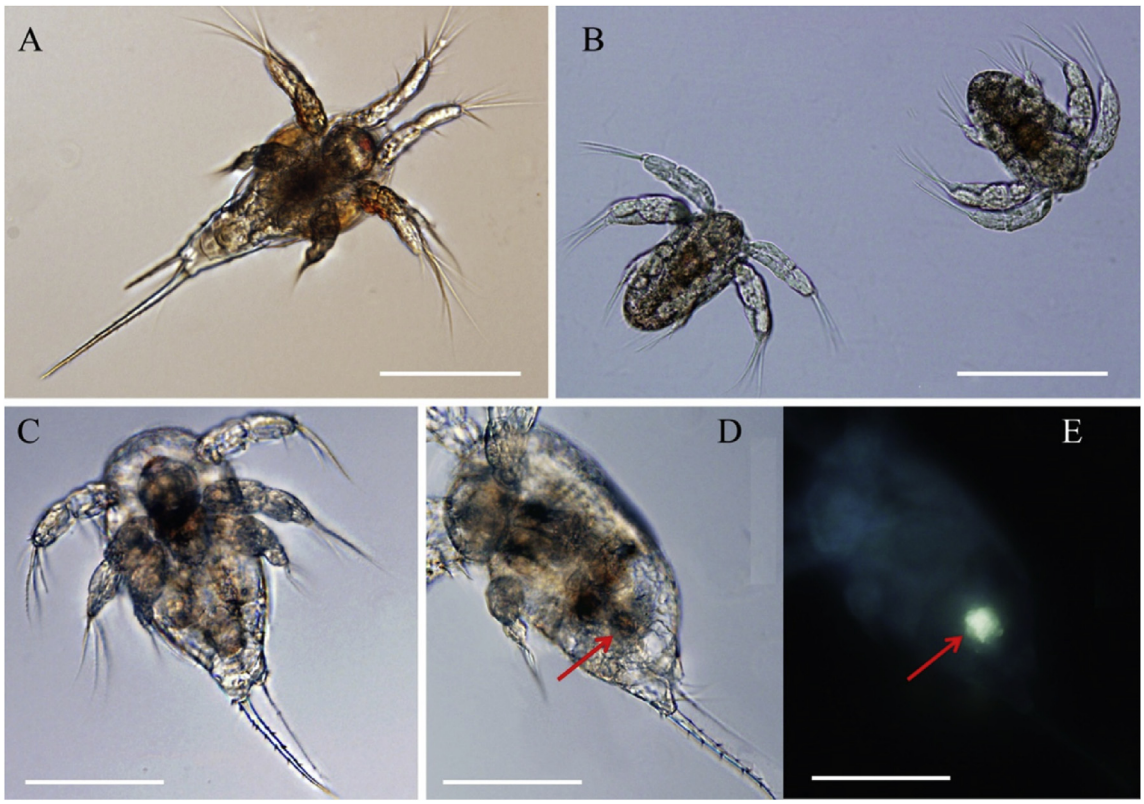


Fig. 1. Microscopy images of naupliar stages of (A) *Temora turbinata* (B) *Acartia tonsa* and (C) *Pseudodiaptomus pelagicus* used as experimental organisms. Images of *P. pelagicus* nauplius with crude oil inside the digestive tract under bright light (D) and UV illumination (E). The presence of crude oil in *P. pelagicus* nauplii was confirmed by autofluorescence of crude oil under UV illumination (E). Scale = 100 μm.

gently sorted from the samples using a borosilicate glass pipette. Isolated copepods were placed into 4–10 L containers with 0.2 μm filtered seawater (FSW, salinity ~35) and kept at 25 °C with aeration. Copepod cultures were fed *ab libitum* every 2 days with a mix of cultured phytoplankton (*Isochrysis galbana*, *Rhodomonas* sp., *Heterocapsa* sp.). Phytoplankton cultures were grown in f/2 culture medium prepared with 0.2 μm filtered sterilized natural seawater collected from the Aransas Ship Channel. Phytoplankton cultures were maintained in 250 mL polycarbonate flasks at 20 °C and 35°/$_{oo}$ salinity on a 12:12 h light:dark cycle with cool-white fluorescent lights at an irradiance of approximately 25 μmol photons m^{-2} s^{-1}.

To obtain copepod nauplii for the exposure experiments, adult copepods were separated from the culture using a 150 μm mesh sieve, placed in a glass beaker containing FSW with aeration and fed *ab libitum* with a mixture of phytoplankton cultures (*Rhodomonas* sp., *Heterocapsa* sp., and *Isochyrsis galbana*). After 24 h, adult copepods were separated from eggs and some hatched nauplii using a 150 μm mesh sieve. Eggs and nauplii were then incubated for another 24–48 h, depending on species, before starting the experiments. Then, copepod nauplii were separated into groups of 20–40 (depending on the experiments, Table 1) using a glass pipette and held in petri dishes with 0.2 μm-FSW until the experiment began (<3 h).

2.2. Preparation of crude oil emulsions

We used Light Louisiana sweet crude oil, which was provided by BP (BP Exploration & Production Inc.), as a surrogate for the crude oil released in the Deepwater Horizon oil spill in the Gulf of Mexico (2010). Corexit 9500A (NALCO®/Exxon Energy Chemicals, L.P., NALCO, 2010a, b), the main type of chemical dispersant used in clean-up operations during the Deepwater Horizon oil spill (National Commission on the BP Deep Ocean Horizon Oil Spill and Offshore Drilling, 2011), was used to prepare chemically dispersed crude oil emulsions.

We used three types of test media: (1) crude oil emulsions, i.e., suspensions of crude oil droplets in seawater dispersed mechanically without the addition of dispersant, (2) dispersant-treated crude oil emulsions, i.e., crude oil emulsions in seawater dispersed mechanically and chemically, and (3) a solution of chemical dispersant alone in seawater. To prepare crude oil emulsions, 1 L of 0.2 μm filtered seawater was placed in a 2 L glass beaker with a magnetic stir bar, which was tightly sealed with aluminum foil to prevent oil absorption on the surface of the bar. The glass beaker containing the seawater was placed on a magnetic stirrer plate and stirred at 900 rpm. Then, 1 mL of crude oil was added to the seawater using an automatic pipette with a Pasteur glass pipette as a tip, that was thoroughly washed to remove the crude oil that could be attach to the pipette tip. After covering the beaker with aluminum foil, the crude oil was emulsified by keeping the stir rate at 900 rpm for 5 min at room temperature (25 °C). This stirring speed caused the formation of a vortex, which extends from the bottom of the container to the water surface, allowing the formation of crude oil droplets in seawater and keeping the crude oil emulsion homogenous during the mixing. To prepare the chemical dispersant treated-oil emulsions, we used the same methodology as for the preparation of the crude oil emulsions, but in this case we added 50 μL of chemical dispersant after adding the crude oil. We used a ratio of dispersant to oil of 1:20 which is in the range recommended by the U.S. Environmental Protection Agency (EPA, 1995). The formation of oil droplets was observed under the microscope and confirmed in previous tests using an Imaging

Table 1
Summary of experimental and environmental conditions during the experiments. We conducted two types of exposure experiments. In the first type of experiments (Exp. # 1 & 2), nauplii of copepods *Acartia tonsa* and *Temora turbinata* were exposed to different concentrations of mechanically dispersed crude oil, ranging from 0.5 to 2 μL L^{-1} with and without UVB radiation. In the second type of experiments (Exp. # 3–5), nauplii of *Pseudodiaptomus pelagicus*, *A. tonsa* and *T. turbinata* were exposed to crude oil alone (1 μL L^{-1}), dispersant (0.05 μL L^{-1}), and dispersant-treated oil (1 μL L^{-1} of crude oil + 0.05 μL L^{-1} of dispersant) with and without UVB radiation. *Stage* indicates the dominant naupliar stage/s at the beginning of the experiments, *L ± SD* is initial average nauplii length ± standard deviation, *Vol.* is volume of the experimental bottles, *Conc.* is concentration of nauplii per bottle, *T* is temperature, *PAR* is Photosynthetically Active Radiation, *UVA* and *UVB* are, respectively, Ultraviolet A and B radiation intensity, and *UVB dose* indicates the total integrated UVB dose after 48 h of exposure to natural sunlight. Note that both mean ± SD and range values of T, PAR, UVA and UVB during sunlight hours (approx. from 8am to 6pm) are indicated to show the variation of environmental factors during the exposure time.

Exp. #	Date	Species	Stage	*L ± SD* (μm)	*Vol.* (mL)	*Conc.* (ind bt^{-1})	*T* (°C)	*PAR* (μmol m^{-2} s^{-1})	*UVA* (μW cm^{-2})	*UVB* (μW cm^{-2})	*UVB dose* (J cm^{-2})
1	July 2013	*A. tonsa*	NIII	141 ± 26	1000	25	31.3 ± 1.5	1688 ± 844	1999 ± 1039	106 ± 63	8.79
							29.1–33.3	410–2930	410–2930	14–186	
2	Oct. 2014	*T. turbinata*	NII	195 ± 24	320	30	27.6 ± 0.7	1140 ± 692	1240 ± 994	104 ± 80	5.40
							26.5–28.6	303–1830	23–2700	7–240	
3	June 2013	*P. pelagicus*	NII	228 ± 24	1000	20	29.2 ± 0.6	1752 ± 663	2018 ± 969	109 ± 63	9.41
							28.0–30.0	752–2636	448–2900	20–197	
4	July 2013	*A. tonsa*	NII	121 ± 15	1000	40	29.7 ± 1.6	1444 ± 824	1545 ± 931	83 ± 56	5.87
							28.2–32.5	282–2432	310–2970	11–164	
5	Oct. 2014	*T. turbinata*	NII–NIII	203 ± 24	320	30	30.8 ± 0.6	1250 ± 627	1385 ± 890	74 ± 60	4.56
							29.8–31.6	360–1870	236–2500	7–153	

Particle Analysis system (FlowSight®) (Almeda et al., 2014c). To prepare the dispersant solutions, 50 μL of chemical dispersant was added to 1 L of 0.2 μm FSW and stirred at 900 rpm for 5 min at 25 °C as in the preparation of the other test media. After the mixing time, aliquots of each test medium were added to the corresponding experimental bottles to obtain the desired exposure nominal concentrations. The total concentration of PAHs in the crude oil used in this study was 2.15 μg μL^{-1} (Almeda et al., 2013b). The specific concentrations and composition of polycyclic aromatic hydrocarbons (PAHs) in this crude oil, including phototoxic PAHs (e.g. fluoranthene, anthracene, pyrene, benzo[a]anthracene and benzo[a] pyrene) can be found in Almeda et al. (2013b).

2.3. Experimental design

We conducted two types of outdoor exposure experiments. In the first type of experiments, copepod nauplii (*A. tonsa*, *T. turbinata*) were exposed to different concentrations of mechanically dispersed crude oil ranging from 0.5 to 2 μL L^{-1} with and without UVB radiation (experiments 1 & 2, Table 1). In the second type of experiments, copepod nauplii (*A. tonsa*, *T. turbinata*, *P. pelagicus*) were exposed to crude oil alone (1 μL L^{-1}), dispersant (0.05 μL L^{-1}), and dispersant-treated oil (crude oil: 1 μL L^{-1}) with and without UVB radiation (experiments 3–5, Table 1). These toxicant exposure concentrations were chosen because they are in the low range of concentrations frequently found in the water column after crude oil spills and/or dispersant applications (McAuliffe et al., 1981; Wells, 1984; Lichtenthaler and Daling, 1985; Clayton et al., 1993; Mukherjee and Wrenn, 2009; National Commission on the BP Deep Ocean Horizon Oil Spill and Offshore Drilling, 2011). In both types of experiments, we included control treatments where copepod nauplii were incubated in absence of pollutants with and without UVB radiation. In all the experiments, control and experimental treatments were run in duplicates (i.e. 2 replicate bottles per treatment) for 48 h. To assess the effect of UVB radiation, exposure experiments were conducted under 2 different light regimes: the full spectrum without UVB (i.e., PAR + UVA, quartz bottles covered with Mylar-D foil) and the full solar radiation spectrum, including UVB (i.e., PAR + UVA + UVB, uncovered quartz bottles). Mylar-D foil is a filter that does not allow the passage of wavelengths <320 nm, i.e., blocks the penetration of UVB (280–315 nm) and allows penetration of UVA (315–400 nm) and PAR (400–700 nm) (Aas et al., 1996).

To begin an experiment, copepod nauplii (20–40, depending on the experiments, Table 1) were gently added to quartz bottles (320–1000 mL) containing 0.2 μm-FSW (salinity = 35 ± 2) and cultured phytoplankton (*Rhodomonas*) at satiation levels (~30,000 cells mL^{-1}). Aliquots of cultured phytoplankton were added to the experimental bottles to obtain the desire food concentrations. The concentration of phytoplankton in the cultures was determined with a microscope using a Sedgewick-Rafter counting chamber or a hemocytometer. At the start of the experiments, two subsamples of 20–40 nauplii were fixed with 1% Lugol's solution for determination of naupliar stage and initial length.

After adding the test media to the corresponding experimental bottles, we covered the bottles for the treatments without UVB with Mylar-D foil. All bottles were incubated on the University of Texas Marine Science Institute pier in a large open/uncovered transparent acrylic container (1.2 m^3) containing a plankton wheel with open-circuit seawater running through it, thus providing similar exposure to sunlight and *in situ* temperature for all the bottles in each experiment. Water temperature, Photosynthetically Active Radiation (PAR), Ultraviolet A (UVA) and UVB radiation intensity, and integrated UVB dose were recorded approximately every 3 h during daylight. Water temperature was measured inside the incubating container and PAR and UV radiation at top the incubation container in the air. Water temperature, PAR and UV radiation were measured using a YSI® Model 30 SCT Meter (and/or a digital thermometer), a LI-COR® LI-250A Light Meter, and a ITL 1700 Research Radiometer, respectively.

An additional test was conducted to examine the presence of crude oil droplets in *P. pelagicus* nauplii guts. Ingestion of crude oil droplets was previously investigated for *A. tonsa* and *T. turbinata* nauplii (Almeda et al., 2014a) but not for *P. pelagicus* nauplii. Twenty *P. pelagicus* nauplii were incubated in 320 mL quartz bottles with food (*Rhodomonas*, 30,000 cells mL^{-1}) and exposed to mechanically dispersed crude oil (1 μL L^{-1}) for 24 h. Bottles were incubated on a Wheaton bench top roller (2–4 rpm) at 25 °C at the laboratory. After incubating, nauplii were collected using a 40 μm mesh sieve, fixed with glutaraldehyde (2%), placed in depression slides, and observed under an epifluorescence microscope with bright-field and UV illumination. The presence of crude oil droplets in the guts of nauplii was verified by the exposure to UV light (365 nm) that produces a strong autofluorescence of crude oil.

2.4. Analysis and calculations

After the 48 h incubation, contents of each bottle (2 replicate bottles per treatment) were gently screened through a submerged 40 μm mesh sieve to collect copepod nauplii and early copepodites.

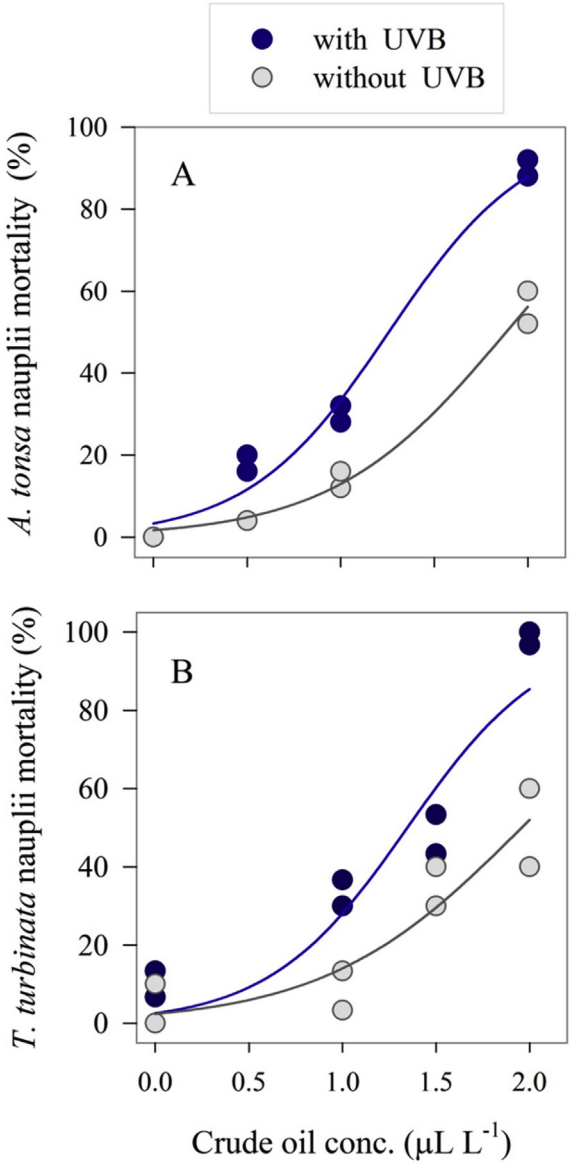


Fig. 2. Relationship between mortality of nauplii of *Acartia tonsa* (A) and *Temora turbinata* (B) and crude oil concentration with and without natural UVB radiation. Regression lines based on Eq. (1). Regression model parameters are indicated in Table 2.

Nauplii and copepodites were placed in glass dishes filled with 0.2 μm-FSW for 5–10 min. We checked the survival of both nauplii and copepodites by gently touching them with a dissecting probe under a stereo microscope. Mortality, as a percent of the total incubated organisms, was estimated from the number of dead copepod nauplii and copepodites at the end of the incubation (48 h). Data on copepod nauplii mortality versus crude oil concentration with and without UVB radiation were fitted to the following sigmoid model:

$$M = 100/\left(1 + e^{-(C-LC_{50})/b}\right) \quad (1)$$

where, M is mortality (%) after 48 h, C is crude oil concentration ($\mu L\,L^{-1}$), LC_{50} is the median lethal concentration ($\mu L\,L^{-1}$) and b is the slope factor.

For *T. turbinata*, after determining survival, live nauplii and copepodites from each treatment and replicate were pipetted into a small glass vessel with filtered seawater to investigate the effects of dispersed crude oil and dispersant on swimming behavior (i.e. length of path, swimming speed, and proportion of time moving). *T. turbinata* nauplii and copepodites from each treatment ($n = 12–45$) were video recorded for 1 min at 30 frames s^{-1} using a Nikon 7100 camera with a Nikon 105 mm F/2.8 FX AF Micro-Nikkor lens and a Fisher Scientific illuminator fiber-optic light source. After filming, the videos were converted from MOV to TIFF files and analyzed using ImageJ and the Particle Tracker 2D/3D Mosaic plugin. This plugin provided automated 2D detection and analysis of individual organism trajectories from recorded videos. After analysis, each trajectory was visually inspected to ensure accurate tracking of each individual copepod nauplii/copepodite. Only the trajectories that were observed to correctly follow a nauplii/copepodite path were kept and any sequence in the trajectory that numerically skipped out of order was deleted to prevent inaccurate speed values. Distance was calibrated using a micrometer in ImageJ using the straight line selection tool. The distance (D, mm) traveled between 2 points (successive video frames) was calculated from the x and y coordinates using the Pythagorean Theorem, as follow:

$$D = \sqrt{\left((x_t - x_{t+1})^2 + (y_t - y_{t+1})^2\right)} \quad (2)$$

where (x_t, y_t) and (x_{t+1}, y_{t+1}) were the positions of copepod nauplii/copepodites at the time t and t+1, respectively. The swimming speed (V, mm s^{-1}) was subsequently estimated as follows:

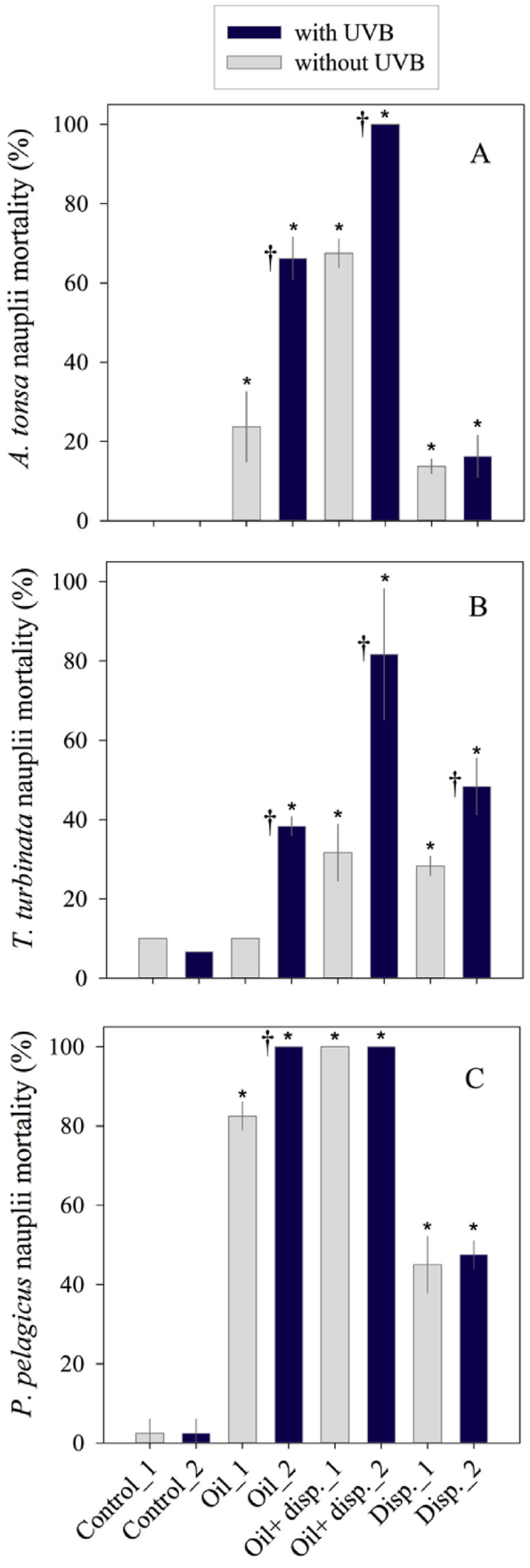
$$V = Df \quad (3)$$

where f is the sampling rate of the camera (30 frames s^{-1}). Average swimming speeds were estimated from each individual swimming trajectory. Path length, i.e., the total distance traveled or gross displacement (mm), was calculated as the sum of the distances (D) traveled during a swimming trajectory. To find the proportion of time in which an individual nauplii/copepodite was moving, the number of times in which the x and y coordinates changed between subsequent video frames (>0 movement) per trajectory was totaled and divided by the number of total frames. Distances less than 0.0018 mm were changed to 0 to account for individuals that were not moving but were given a value greater than 0 in ImageJ. To avoid large values due to incorrect path tracking, any distance over 4 mm was not considered. Both 0.0018 and 4 mm were chosen based on the histogram distribution of movement data (data not shown).

After determining survival and swimming behavior, copepod nauplii and copepodites were fixed with Lugol's solution (1%) for growth determination. Pictures of all copepod larvae from each treatment and replicate, and from the initial samples were taken with an Olympus DP26 digital camera attached to a stereomicroscope using Olympus CellSens Digital Imaging software. Nauplii body length, excluding caudal setae or spines, and copepodite prosome length were measured using image analysis (ImageJ

Table 2
Parameters for the sigmoidal model (equation (1), Fig. 2) used to describe the relationship between mortality of *Acartia tonsa* and *Temora turbinata* nauplii and crude oil concentration with (+) or without (−) UVB radiation. LC_{50}: median lethal concentrations, SE: standard error, b: slope factor, r^2: correlation coefficient, F: F statistic (from ANOVA) and p: significance level.

Species	UVB	$LC_{50} \pm SE$ ($\mu L\,L^{-1}$)	$b \pm SE$	r^2	F	p
A. tonsa	−	1.88 ± 0.03	0.46 ± 0.03	0.98	338.9	<0.0001
	+	1.25 ± 0.05	0.37 ± 0.04	0.98	479.7	<0.0001
T. turbinata	−	1.95 ± 0.12	0.52 ± 0.14	0.84	32.2	0.0013
	+	1.34 ± 0.09	0.36 ± 0.09	0.89	51.7	0.0004



software). To calculate specific growth rates, carbon content of nauplii and copepodites was calculated from length-weight regressions described in Berggren et al. (1988) for *A. tonsa*, Robert et al. (2011) for *T. turbinata* (as for *Temora longicornis*) and Uye et al. (1983) for *P. pelagicus* (as for *Pseudodiaptomus marinus*). C-specific growth rate (G, d^{-1}) in each treatment replicate was calculated as

$$G = (\ln\ (W_2/W_1))/t \quad (4)$$

where W_2 and W_1 are the mean carbon weight (ng-C) at the end and the beginning of the incubation, respectively, and t is the incubation time in days.

For statistical analyses, we used one way analysis of variance (ANOVA) to determine significant differences among treatments and post hoc Dunnett's test for comparison of the different experimental treatments with the corresponding control. When data did not follow a normal distribution (Shapiro–Wilks test), data were normalized using square root transformation. Two replicates per treatment were used for comparisons of mortality and growth rates (n = 2 per treatment). For comparisons of swimming behavior parameters, nauplii from both replicate bottles were combined and each individual nauplii was considered as a replicate (n = 12–45 per treatment). A significance level (p) of 0.05 was considered.

3. Results

3.1. Ingestion of dispersed crude oil by copepod nauplii

We detected ingestion of dispersed crude oil by *P. pelagicus* nauplii after exposure to crude oil emulsions (Fig. 1D–E) as we previously observed in naupliar stages of *A. tonsa* and *T. turbinata* [34]. The presence of crude oil droplets inside the digestive tract of *P. pelagicus* nauplii was unequivocally confirmed by the strong autofluorescence of crude oil under UV light (Fig. 1E).

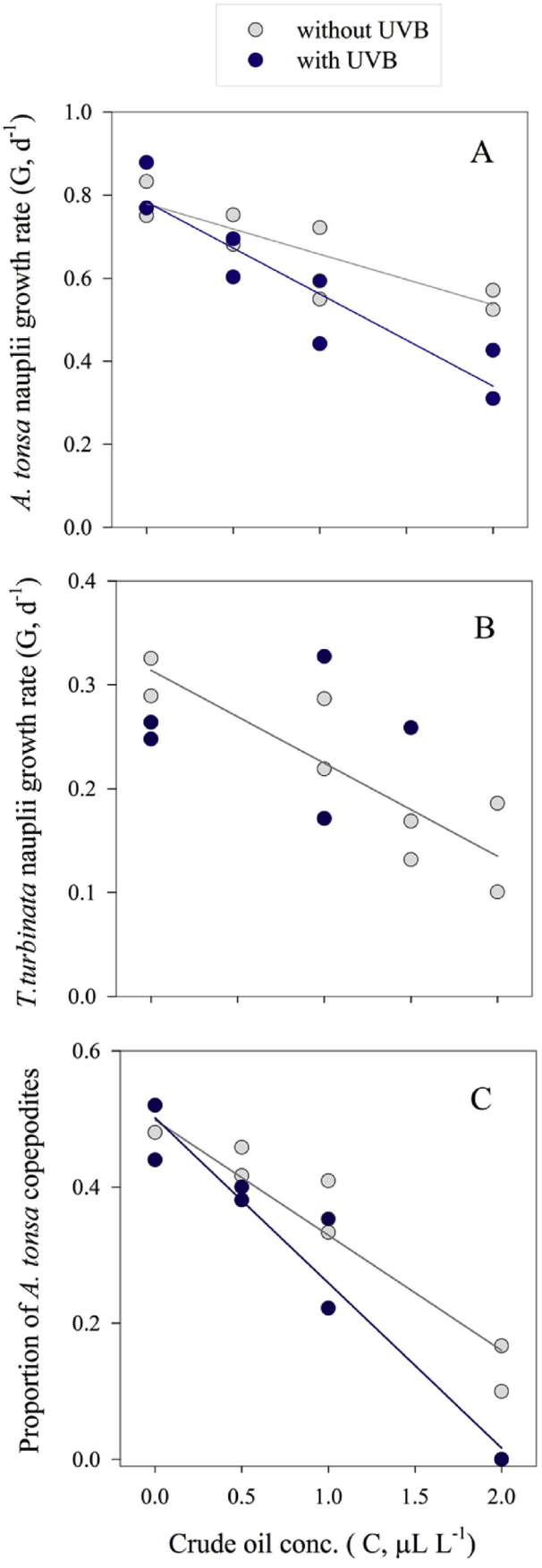
3.2. Environmental conditions during the experiments

Environmental conditions were similar for all bottles in any single experiment but varied among the different exposure experiments (Table 1). Mean temperature and PAR ranged from 27.6 to 31.3 °C and from 1250 to 1752 μmol m^{-2} s^{-1}, respectively, depending on the experiment (Table 1). Mean UVA radiation intensity (range: 1240–2018 μW cm^{-2}) was 1–2 orders of magnitude higher than UVB radiation intensity (range: 74–109 μW cm^{-2}) (Table 1). Total integrated UVB dose after 48 h of exposure to natural sunlight in the experiments ranged from 4.56 to 9.41 J cm^{-2}, with the maximum value observed in June 2013 (exp. 3, Table 1).

3.3. Effect of UVB radiation on the lethal toxicity of dispersed crude oil to copepod nauplii

Copepod nauplii mortality (%) increased with increasing dispersed crude oil concentration, reaching values >80% for both *A. tonsa* and *T. turbinata* at a crude oil concentration of 2 μL L^{-1} after 48 h in presence of UVB radiation (Fig. 2). The patterns in the relationship between naupliar mortality (%) and dispersed crude oil concentration were relatively similar for both copepod species

Fig. 3. Influence of UVB radiation on the lethal effect of crude oil alone ("Oil", 1 μL L-1), dispersant-treated oil ("Oil + Disp.", 1 μL L^{-1} of crude oil + 0.05 μL L^{-1} of dispersant) and dispersant alone ("Disp.", 0.05 μL L^{-1}) on nauplii of *Acartia tonsa* (A), *Temora turbinata* (B) and *Pseudodiaptomus pelagicus* (C). Error bars are standard deviation. Asterisks indicate significantly lower than the controls (ANOVA, Dunnet test, $p < 0.05$). Dagger (†) indicates a statistically significant effect of UVB radiation (ANOVA, $p < 0.05$).



(Fig. 2) and were well described by the sigmoid model (r^2 = 0.84–0.98) (Fig. 2, Table 2). According to the model, median lethal concentration (LC_{50}) for *A. tonsa* and *T. turbinata* nauplii exposed to crude oil without UVB radiation was 1.88 and 1.95 µL L^{-1}, respectively (Table 2). In the presence of UVB radiation, LC_{50} for *A. tonsa* and *T. turbinata* nauplii were, respectively, 1.25 and 1.34 µL L^{-1}, which implies an increase in lethal toxicity of crude oil by ~1.5 times compared to treatment without UVB radiation (Table 2).

Mortality of copepod nauplii after 48 h of exposure to crude oil alone (1 µL^{-1}), dispersant treated-crude oil (1 µL^{-1}) and dispersant (0.05 µL^{-1}) was significantly higher than in the controls under both light regimes for *A. tonsa* (without UV: ANOVA, $F_{3,4}$ = 292.19, $p < 0.001$, Dunnet test, $p < 0.005$; with UV: ANOVA, $F_{3,4}$ = 97.54, $p < 0.001$, Dunnet test, $p < 0.005$) (Fig. 3A) and for *P. pelagicus* (without UV: ANOVA, $F_{3,4}$ = 45.18, $p < 0.002$, Dunnet test, $p < 0.005$; with UV: ANOVA, $F_{3,4}$ = 54.68, $p = 0.001$, Dunnet test, $p < 0.001$) (Fig. 3C). *T. turbinata* nauplii showed higher mortality in the experimental treatments than in the controls (without UV: ANOVA, $F_{3,4}$ = 31.30, $p = 0.003$, with UV: ANOVA, $F_{3,4}$ = 51.13, $p = 0.001$, Dunnet test, $p < 0.001$) except for the treatment with crude oil alone without UVB where no significant difference was observed (Dunnet test, $p > 0.05$) (Fig. 3B). Mortality of copepod nauplii was ≤10% in the controls, whereas it ranged from 10 to 100% in the experimental treatments (Fig. 3). Depending on copepod species, UVB radiation significantly increased mortality by 1.3–3.8 times when nauplii were exposed to crude oil alone (*A. tonsa*: ANOVA, $F_{1,2}$ = , $p = 0.042$; *T. turbinata*: ANOVA, $F_{1,2}$ = 543.24, $p = 0.002$; *P. pelagicus*: ANOVA, $F_{1,2}$ = 44.25, $p = 0.022$) and by 1.5–2.9 times when nauplii were exposed to dispersant-treated oil (*A. tonsa*: ANOVA, $F_{1,2}$ = 136.11, $p = 0.007$; *T. turbinata*: ANOVA, $F_{1,2}$ = 18.74, $p = 0.049$) (Fig. 3). UVB did not affect mortality of *A. tonsa* and *P. pelagicus* nauplii when exposed to dispersant alone but increased *T. turbinata* nauplii mortality by 1.7 times, although not statically significant (ANOVA, $F_{1,2}$ = 16.91, $p = 0.054$) (Fig. 3B). The percent reduction in naupliar survival (relative to the controls) was higher in the dispersant-treated oil treatment than in the other experimental treatments for all three species, particularly in presence of UVB (80–100%, Table 4). At a dispersant to crude oil ratio of 1:20, lethal effects of dispersant alone (0.05 µL L^{-1}) were lower than crude oil alone (1 µL L^{-1}) for *A. tonsa* and *P. pelagicus*. On the contrary, differences in survival from the controls was greater with dispersant alone (0.05 µL L^{-1}) than crude oil alone (1 µL L^{-1}) for *T. turbinata* nauplii (Table 4). Among species and all experimental treatment, *P. pelagicus* nauplii had the highest percent decrease for survival, except when *A. tonsa* nauplii was exposed to dispersant treated-oil with UVB and the percent decrease in survival was similar to *P. pelagicus* nauplii (100%, Table 4).

3.4. Sublethal effects of dispersed crude oil to copepod nauplii and the influence UVB radiation.

Specific growth rates nauplii of *A. tonsa* and *T. turbinata* significantly decreased as dispersed crude oil concentration increased (Fig. 4A–B) (Table 3). The proportion of *A. tonsa* copepodites at the end of the incubation (48 h) was also negatively correlated with dispersed crude oil concentration (Fig. 4C) (Table 3). The number of

Fig. 4. C-specific naupliar growth rates of *Acartia tonsa* (A) and *Temora turbinata* (B), and proportion of *A. tonsa* copepodites at the end of the incubation (C) after exposure to different concentrations of crude oil with or without natural UVB radiation after 48 h of exposure. Regression parameters are indicated in Table 3. Note that in (B) we could not determine growth at 2 µL L^{-1} (since mortality was ~100%) and one replicate for 1 µL L^{-1} was lost which precludes any clear trend for the treatment with UVB.

Table 3
Parameters for the linear ($y = y_0 - bC$, Fig. 4) and exponential decay ($y = y_0{}^{*}e^{-bC}$, Fig. 5) regressions used to describe the relationship between copepod nauplii sublethal endpoints (y) and crude oil concentration (C) with (+) and without (−) UVB radiation or combining both light treatments (−/+), when there was no significant difference between light treatments. *b*: slope factor, y_0: intercept (endpoint value when x = 0, i.e., in absence of crude oil), SE: standard error, r^2: correlation coefficient, F: F statistic (from ANOVA) and *p*: significance level.

Endpoints	Species	UVB	*b* ± SE	y_0 ± SE	r^2	F	*p*
Growth rate (d^{-1})	*Acartia tonsa*	−	−0.12 ± 0.03	0.78 ± 0.03	0.74	16.6	0.0065
		+	−0.22 ± 0.04	0.78 ± 0.04	0.85	33.7	0.0011
	Temora turbinata	−	−0.09 ± 0.02	0.31 ± 0.03	0.76	19.4	0.0045
Frequency of copepodites	*Acartia tonsa*	−	−0.17 ± 0.02	0.49 ± 0.03	0.88	47.1	0.0005
		+	−0.24 ± 0.02	0.50 ± 0.03	0.94	101.3	<0.0001
Swmming speed (mm s^{-1})	*Temora turbinata*	−/+	−0.44 ± 0.09	0.70 ± 0.07	0.79	19.0	0.0073
Path length (mm)	*Temora turbinata*	−/+	1.30 ± 0.15	1.69 ± 0.19	0.93	64.9	0.0005

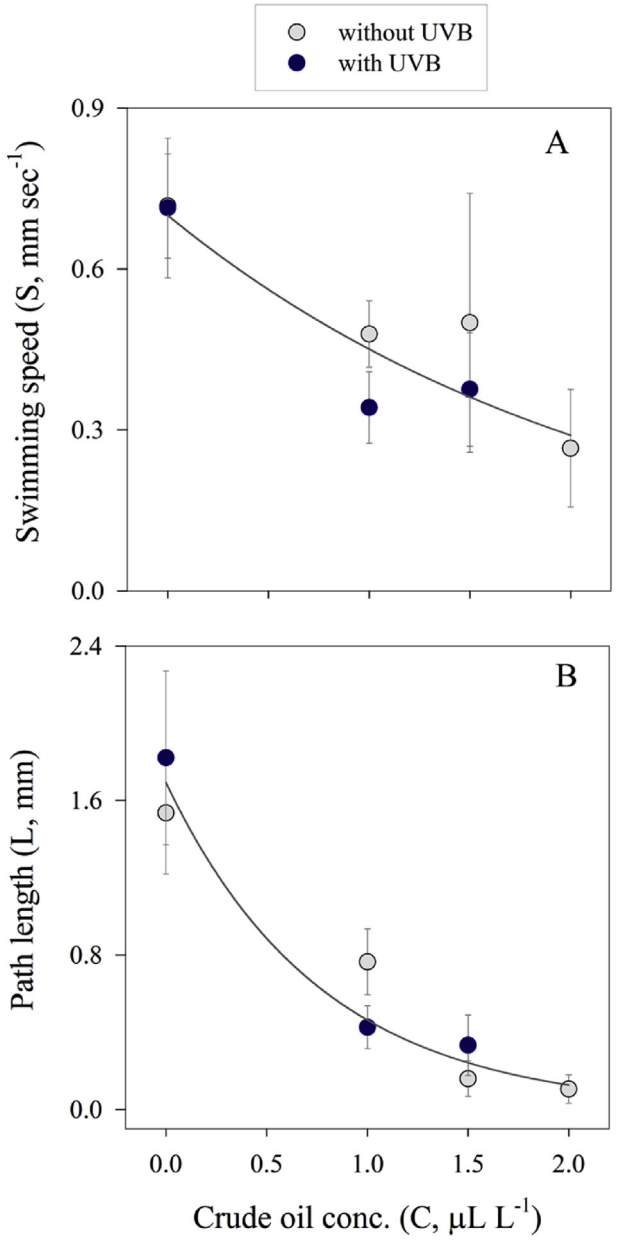


Fig. 5. Effect of mechanically dispersed crude oil concentration on swimming speed (A) and path length (B) of *Temora turbinata* nauplii with or without natural UVB radiation after 48 h of exposure. Regression parameters are indicated in Table 4. Error bars are standard errors. Note that data from both light regimes were combined in the fitted regression curves because there was no significant difference between UVB treatments in this case.

T. turbinata copepodites at the end of the incubation was low (in average < 10%) with no significant difference among treatments. Swimming speed and path length of *T. turbinata* nauplii decreased exponentially when exposed to increasing concentrations of crude oil without a clear influence of UVB (Fig. 5) (Table 3). We did not find a decrease in the percent of time moving of *T. turbinata* nauplii in relation to crude oil concentration.

Exposure to crude oil (1 µL L^{-1}), chemical dispersant alone (0.05 µL^{-1}) or dispersant-treated oil (1 µL L^{-1}) decreased specific growth rates of *A. tonsa* and *T. turbinata* nauplii (Fig. 6) and swimming behavior parameters (i.e., proportion of time moving, swimming speed, and length path) of *T. turbinata* nauplii (Fig. 7) compared to the controls. Growth rates of *P. pelagicus* could not be determined due to the high mortality in the experimental treatments. The proportion of *A. tonsa* copepodites at the end of the incubation was drastically reduced when nauplii were exposed to crude oil and dispersant-treated oil (Fig. 6B). In contrast, naupliar growth rates and frequency of copepodites of *T. turbinata* at the end of the incubation were less affected by the studied pollutants compared to *A. tonsa* (Fig. 6). Naupliar growth rates were lower in the presence of UVB than without UVB, although not statistically significant (ANOVA, $p > 0.05$), except for *T. turbinata* nauplii exposed to dispersant-treated oil, where growth rates were significantly lower, by 1.6-fold, with UVB than without UVB (ANOVA, $F_{1,2} = 20.76$, $p = 0.045$) (Fig. 6). The percent reduction in naupliar growth rates when exposed to crude oil and/or dispersant compared to the controls ranged from 14 to 100% depending on species and experimental treatments (Table 4). Among experimental treatments, the greatest percent reduction in naupliar growth rates (46% for *T. turbinata*, 100% for *A. tonsa*) and swimming behavior measurements (63–73%) compared to the controls was observed when nauplii were exposed to dispersant-treated oil in the presence of UVB (Table 4). The percent reduction in time moving, swimming speed, and length path of *T. turbinata* nauplii exposed to dispersant-treated oil was higher in presence of UVB, specifically, 2.3, 2.4 and 3.1 times higher, respectively (Table 4).

4. Discussion

Our results demonstrate that exposure to dispersed crude oil and chemical dispersant at concentrations generally found in the sea after oil spills are toxic to copepod nauplii and that natural UVB radiation levels substantially increase the toxicity of crude oil to these planktonic organisms. Acute exposure to dispersed crude oil in the presence of UVB caused harmful effects on copepod nauplii at a concentration of ~1 ppm, which indicates that dispersed crude oil is highly toxic for these zooplankters according to U.S. Environmental Protection Agency's five-step scale of toxicity categories of pesticides (EPA, 2015). Similarly, we observed that a low concentration of chemical dispersant Corexit 9500 (~0.05 ppm) caused harmful effects on copepod nauplii, suggesting that this type of

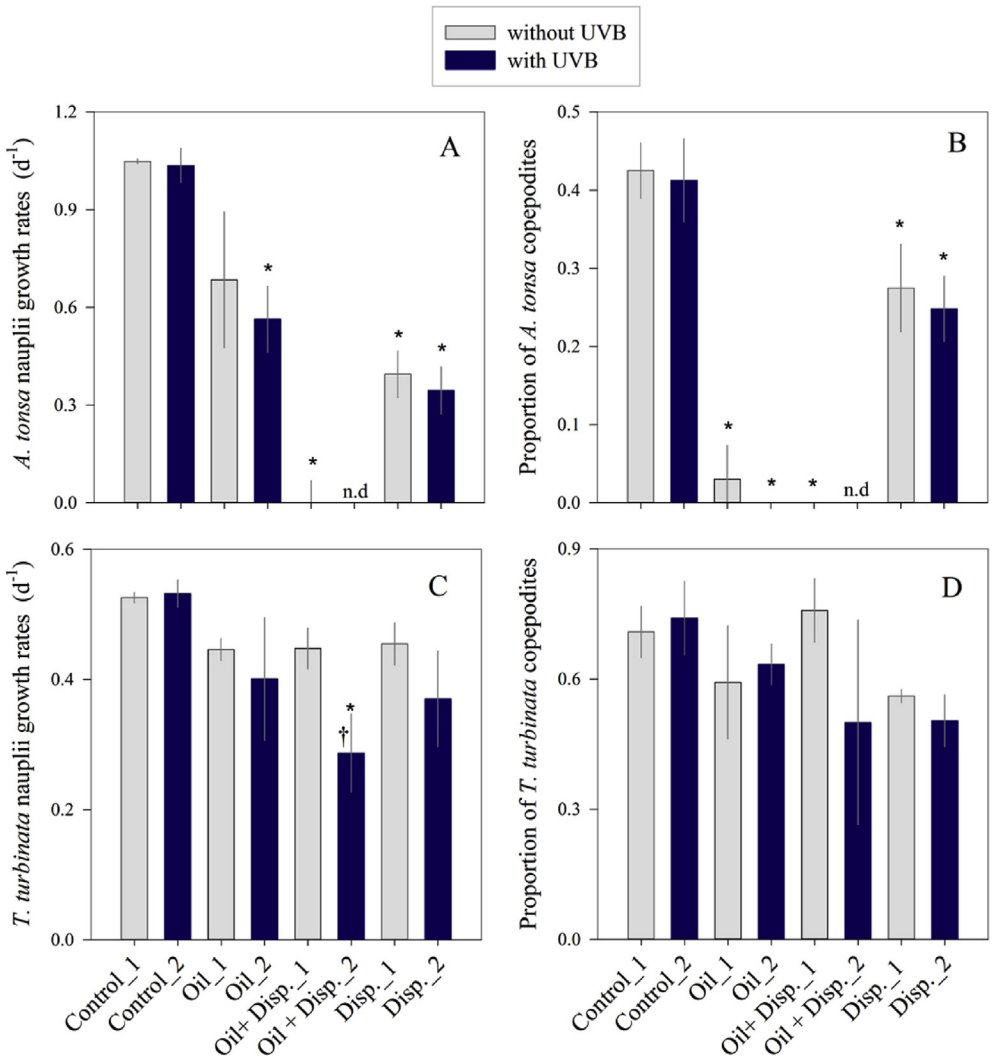
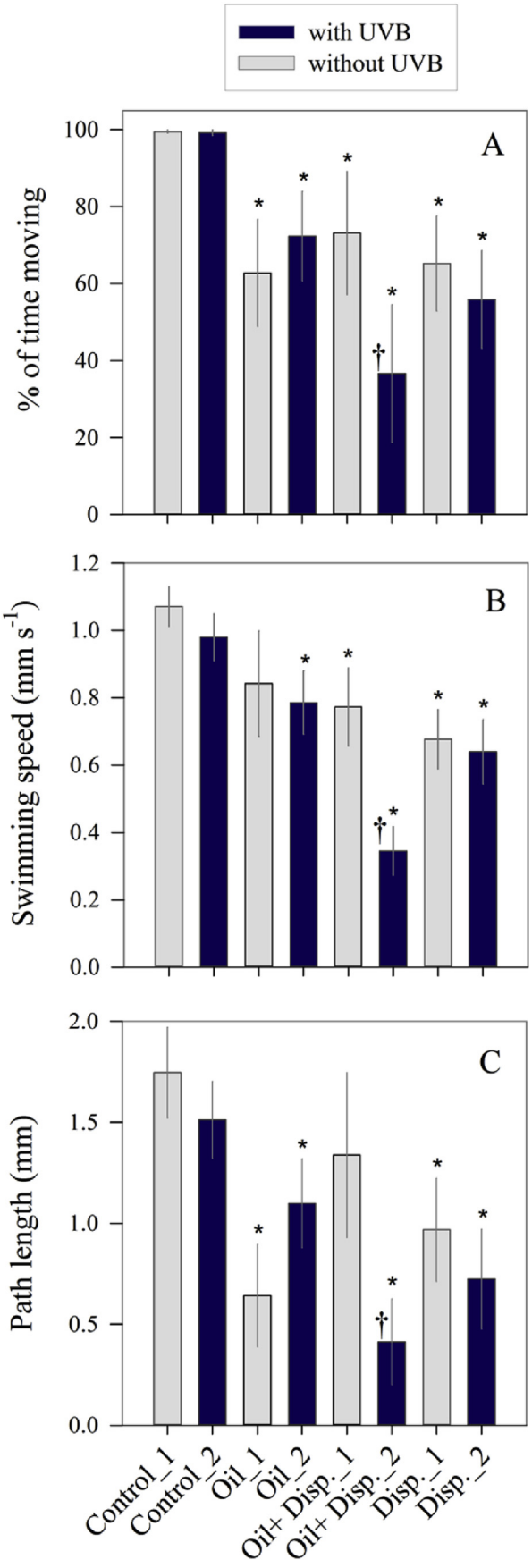


Fig. 6. C-specific growth rates and proportion of copepodites at the end of the incubation of *Acartia tonsa* (A–B) and *Temora turbinata* (C–D) after 48 h of exposure to crude oil alone ("Oil", 1 μL L^{-1}), dispersant-treated oil ("Oil + Disp.", 1 μL L^{-1} of crude oil + 0.05 μL L^{-1} of dispersant) and dispersant alone ("Disp.", 0.05 μL L^{-1}) with or without natural UVB radiation. Error bars represent standard deviations. Asterisks indicate significantly lower than the controls (ANOVA, Dunnet test, $p < 0.05$). Dagger (†) indicates a statistically significant effect of UVB radiation (ANOVA, $p < 0.05$). n.d.: indicates experimental treatments where mortality was 100% and growth rates could not be determined.

dispersant also falls in the very high toxicity category using the same EPA toxicity category scale (EPA, 2015). A previous study found that mortality of adult stages of *Acarti tonsa* and *T. turbinata* was ~26% and 7%, respectively, after 48 h of exposure to 1 μL L^{-1} of dispersant-treated oil in absence of UV radiation (Almeda et al., 2014a). In comparison, mortality of *A. tonsa* and *T. turbinata* nauplii was ~2–3 -fold higher than mortality of their adult stages at similar exposure conditions (Almeda et al., 2014a). This indicates that early larval stages of copepods are more sensitive to dispersed oil than adults of the same species, as frequently observed in other marine animals (Goodbody-Gringley et al., 2013; Almeda et al., 2013b, 2014b). Our results support previous studies that indicate that small zooplankton, especially early-life stages and ciliates, are particularly sensitive to dispersed crude oil and chemical dispersants (George-Ares and Clark, 2000; Goodbody-Gringley et al., 2013; Almeda et al., 2013b, b, d) and therefore, highly vulnerable to the impact of oil spills and dispersant applications in marine environments. Negative effects of dispersed oil on copepod nauplii survival may affect fish production since copepod nauplii are the main food of many fish larvae and their abundance determines the recruitment of commercially important fish species (Last, 1980; Castonguay et al., 2008). Additionally, copepod nauplii are important grazers in marine plankton food webs (Böttjer et al., 2010; Almeda et al., 2011) and the effects of dispersed crude oil on nauplii abundance could affect phytoplankton and microbial communities. Therefore, harmful impact of dispersed crude oil on copepod nauplii would likely affect plankton population dynamics and secondary production in marine environments.

We found that low concentrations of the studied pollutants caused not only lethal effects on copepod nauplii but also deleterious sublethal effects, such as reduced growth, development and swimming activity. Previous studies have also found reduced growth in meroplanktonic larvae, and reduced egg production and hatching in *T. turbinata* and *A. tonsa* after exposure to similar types and concentrations of crude oil and dispersants (Almeda et al., 2014a, b). Altered swimming behavior of copepods exposed to dissolved petroleum hydrocarbons has been observed in adult stages, with effects depending on species and gender (Seuront and Leterme, 2007; Seuront, 2010, 2012; Cohen et al., 2014). Petroleum hydrocarbons may enter into nervous tissue and functionally disrupt the nervous system (Barron et al., 2001), resulting in narcosis (Berdugo et al., 1977; Barata et al., 2005; Calbet et al., 2007)



and affecting swimming activity. In calanoid copepods, feeding and swimming are directly linked, where amount of time moving, swimming speed and length path are associated with encounter rates of prey (Van Duren and Videler, 1996; Titelman and Kiørboe, 2003a). Therefore, the observed decrease in swimming activity in copepod nauplii after exposure to crude oil can lead to reduced feeding efficiency, and consequently reduced growth rates. Also, the ingestion of crude oil droplets by copepod nauplii may decrease assimilation efficiency and negatively affect growth. Other physiological impacts after exposure to petroleum hydrocarbons, such as alterations in steroid metabolism, may also negatively affect larval development of crustaceans (Singer and Lee, 1977; Capuzzo et al., 1984). Therefore, effects of dispersed crude oil on swimming/feeding, energetics and biochemical processes may explain the reduced growth rates of copepod nauplii observed in this study. From an ecological perspective, the observed alterations in swimming may reduce the ability of copepod nauplii to escape predators, which would result in greater mortality from predation in nature (Titelman, 2001; Buskey et al., 2002; Titelman and Kiørboe, 2003b). Additionally, slower development rate increases exposure time of nauplii to predators and reduces the probability of metamorphic success into adult/reproductive stages. Negative sublethal effects of dispersed oil during early developmental stages may also affect the performance of subsequent life stages. Therefore, besides direct lethal effects, sublethal effects of dispersed crude oil on copepod nauplii may also have important implications on recruitment and population dynamics of planktonic copepods.

Effects of UVB radiation on marine life have been an important concern since discovery that the ozone layer has thinned and that UVB radiation levels reaching Earth's surface have increased (Madronich et al., 1998; McKenzie et al., 2007). Although UVB radiation can cause direct damage at molecular, cellular and organismal levels (Tevini, 1993; Browman et al., 2000), harmful effects of UVB radiation alone on copepod nauplii were not observed in our experiments, even at high UVB doses. Copepod species from the Gulf of Mexico, where UV radiation levels are naturally high, may be adapted to tolerate the UVB exposure levels received during our experiments. Conversely, UVB radiation in combination with dispersed crude oil had a significant negative impact on copepod nauplii, as previously observed in other marine organisms when exposed to dissolved petroleum hydrocarbons (Boese et al., 1997; Pelletier et al., 1997; Barron et al., 2003; Duesterloh et al., 2003) or dispersed crude oil (Almeda et al., 2013a). A previous study reported that photoenhanced toxicity on fish larvae occurred only when oil was present in the tissues, suggesting that photosensitization of bioaccumulated petroleum compounds was the main phototoxicity mechanism (Barron et al., 2003). Ingestion of crude oil droplets by copepod nauplii observed here and in a previous study (Almeda et al., 2014a) could enhance photosensitization. Crude oil droplets inside nauplii guts may act directly as photoreceptors of UVB and transfer radiation energy to other surrounding biomolecules, causing damage in the gut cells. Also, after ingestion of crude oil droplets, petroleum hydrocarbons may be absorbed during the gut transit, and subsequently photosensitization of accumulated petroleum hydrocarbons may occur. We suggest that ingestion of oil droplets, besides promoting the entry of toxic petroleum compounds into marine food webs, could increase

Fig. 7. Percent of time swimming (A), swimming speed (B) and path length (C) of *Temora turbinata* nauplii C after 48 h of exposure to crude oil alone ("Oil", 1 μL L^{-1}), dispersant-treated oil ("Oil + Disp.", 1 μL L^{-1} of crude oil + 0.05 μL L^{-1} of dispersant) and dispersant alone ("Disp.", 0.05 μL L^{-1}) with or without natural UVB radiation. Error bars represent the standard error. Asterisks indicate significantly lower than the controls (ANOVA, Dunnet test, $p < 0.05$). Dagger (†) indicates a statistically significant effect of UVB radiation (ANOVA, $p < 0.05$).

Table 4
Percent reduction (mean ± standard error) in naupliar survival, specific growth rates, proportion of copepodites at the end of the incubation, and swimming behavior measurements in the experimental treatments (i.e., "Oil" = 1 μL L^{-1} of crude oil alone, "Oil + Disp." = 1 μL L^{-1} of crude oil + 0.05 μL L^{-1} of dispersant, and "Disp." = 0.05 μL L^{-1} of dispersant alone) compared to the controls with (+) and without (−) UVB radiation. Asterisks indicate significantly different than the controls (ANOVA, $p < 0.05$). n.d.: indicates experimental treatments where mortality was 100% and sublethal effects could not be determined.

Endpoints	Species	UVB	Percent reduction (%)		
			Oil	Oil + Disp.	Disp.
Survival (%)	*A. tonsa*	−	24 ± 9*	68 ± 4*	14 ± 2*
		+	66 ± 5*	100 ± 0*	15 ± 7*
	T. turbinata	−	0 ± 0	24 ± 8*	20 ± 3*
		+	34 ± 3*	80 ± 18*	43 ± 8*
	P. pelagicus	−	82 ± 4*	100 ± 0*	44 ± 7*
		+	100 ± 0*	100 ± 0*	41 ± 4*
Growth rate (d^{-1})	*A. tonsa*	−	38 ± 29*	100 ± 6*	62 ± 7*
		+	51 ± 19*	n.d.	66 ± 7*
	T. turbinata	−	15 ± 3*	15 ± 6	14 ± 6
		+	25 ± 18	46 ± 11*	30 ± 14
Proportion of copepodites	*A. tonsa*	−	93 ± 10*	100 ± 0*	35 ± 15*
		+	100 ± 0*	n.d.	40 ± 10*
	T. turbinata	−	16 ± 18	0 ± 10	21 ± 2
		+	14 ± 6	32 ± 32	32 ± 2
Time spent moving (%)	*T. turbinata*	−	37 ± 14*	26 ± 16*	34 ± 12*
		+	27 ± 12*	63 ± 18*	44 ± 13*
Swmming speed (mm s^{-1})	*T. turbinata*	−	21 ± 15	28 ± 11*	37 ± 8*
		+	20 ± 10*	65 ± 7*	35 ± 10*
Path length (mm)	*T. turbinata*	−	63 ± 15*	23 ± 23	45 ± 15*
		+	27 ± 15*	73 ± 14*	52 ± 16*

phototoxicity of crude oil to zooplankton. Further studies are required to better evaluate the importance of the different mechanisms causing oil phototoxicity and the influence of ingestion of crude oil on photoenhanced toxicity of crude oil to zooplankton.

Enhanced toxicity of petroleum to marine animals due to UV radiation varies widely (from 1.3 to >100 times), depending on many factors such as type of petroleum (single PAH, WAF, or crude oil), UV dose, use of chemical dispersants, exposure time, and species (Boese et al., 1997; Pelletier et al., 1997; Calfee et al., 1999; Barron et al., 2003; Duesterloh et al., 2003; Bejarano et al., 2006; Cleveland et al., 2000; Incardona et al., 2012; Almeda et al., 2013a). In this study, UVB increased the toxicity of crude oil by a similar factor (~1.3–3.8 times) to those frequently observed for other small aquatic crustaceans when exposed to WAF (1.3–10 times) (Calfee et al., 1999; Duesterloh et al., 2003; Bejarano et al., 2006; Cleveland et al., 2000). A previous study with mesozooplankton communities from the Gulf of Mexico (mainly adult copepods) exposed to similar dispersant-treated oil as used in this study found that lethal toxicity was 1.5 times greater in presence of UVB (Almeda et al., 2013a), which is similar to the increase in mortality (LC_{50}) observed for copepod nauplii in this study. Among the nauplii species studied here, we found that toxicity of crude oil and phototoehanced toxicity was similar for *A. tonsa* and *T. turbinata* nauplii, despite differences in UVB dose (Table 1), indicating that the UVB received in autumn (4.56 J cm^{-2}) was enough to cause effective phototoenhaced toxicity to copepod nauplii. In fact, photoenhanced toxicity in aquatic organisms can occur at very low UV intensities (1% of the surface level of UV) (Little et al., 2000; Barron and Ka'aihue, 2001). Then, although UV exposure on marine organisms in the water column can greatly vary due to biological and abiotic factors (e.g. light attenuation) (Little et al., 2000; Barron and Ka'aihue, 2001; Pelletier et al., 2006), we expect that photoenhanced toxicity of crude oil occurs in the photic zone of the water column after oil spills, particularly in surface waters where crude oil accumulates and light radiation is high. Therefore, planktonic organisms, especially those adapted to live in the upper layers of the water column and in intertidal and shallow coastal waters would be particularly vulnerable to crude oil phototoxicity after oil spills.

We clearly found that interactions among crude oil, chemical dispersant and UVB radiation resulted in greater toxic effects on copepod nauplii, as previously observed on fish larvae exposed to WAF (Barron et al., 2003). Increased toxicity of dispersant-treated oil compared to crude oil alone can result from additive and/or synergistic effects of crude oil and chemical dispersant. As demonstrated here and in previous studies, Corexit 9500A dispersant is itself toxic to marine zooplankton (Goodbody-Gringley et al., 2013; Almeda et al., 2013a, 2014a, b, d). The toxic mechanisms of chemical dispersants are not fully known but they can increase permeability of cellular membranes, causing membrane lysis in marine organisms (Nagel et al., 1974; Singer et al., 1990). Besides its own toxicity, chemical dispersant may promote dissolution of PAHs (Greer et al., 2012; Wu et al., 2012) into the aqueous phase. PAHs are the most phototoxic compounds of crude oil (Pelletier et al., 1997) and dissolution of PAHs can facilitate their bioavailability and bioaccumulation in tissues, and consequently enhance photosentization in the presence of sunlight/UVB (Barron et al., 2003). Although the impact of UVB radiation on lethal toxicity was similar for crude oil alone and dispersant-treated oil, photoenhanced sublethal toxicity (on growth and swimming behavior) was significantly higher when *T. turbinata* nauplii were exposed to dispersant-treated oil than to crude oil alone, suggesting that chemical dispersion of crude oil can promote photoenahcend toxicity of crude oil to marine zooplankton, as indicated in previous studies (Barron and Ka'aihue 2001; Barron et al., 2003). Therefore, a negative consequence of using chemical dispersants after marine oil spills would be that dispersants will foster photoenhanced toxicity of crude oil to marine animals. More research is required to accurately determine if chemically dispersed crude oil is more phototoxic than non-chemically dispersed oil to marine zooplankton. Overall, our results indicate that the application of chemical dispersants (Corexit 9500) after oil spills may increase the damage to key zooplankton organisms, such as copepod nauplii, and consequently affect negatively marine planktonic systems.

Standard laboratory toxicity tests are a fundamental tool to understand the effects of pollutants on marine zooplankton, but direct extrapolation to the field needs to be taken cautiously. In the natural environment, the impact of oil spills on zooplankton will

vary widely depending on multiple factors, including sunlight. Excluding sunlight in petroleum toxicological studies can substantially underestimated the impact of crude oil spills on zooplankton, particularly on early-live stages adapted to live in the upper layers of the water column and/or intertidal and shallow coastal waters. Overall, our results emphasize the importance of including sunlight in petroleum toxicological studies and models to estimate better the potential impact of oils spills on marine zooplankton.

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